Genetics of LDL Subclass Phenotypes in Women Twins
Concordance, Heritability, and Commingling Analysis

Melissa A. Austin, Beth Newman, Joe V. Selby, Karen Edwards, Elizabeth J. Mayer, and Ronald M. Krauss

Low density lipoprotein (LDL) subclass phenotype B, characterized by a predominance of small LDL as determined by gradient gel electrophoresis, has been associated with increased risk of coronary heart disease and an atherogenic lipoprotein profile. Previous studies employing complex segregation analysis have demonstrated a major, single gene effect on the inheritance of this phenotype in families. Recently, linkage between this phenotype and variation at the LDL receptor locus on chromosome 19 has been reported. However, variation in LDL subclass phenotypes has also been associated with age, gender, diabetes status, β-blocker medication, and diet. The present study further evaluates the relative importance of genetic and nongenetic influences on LDL subclass phenotypes and on LDL peak particle diameter (as a reflection of the size of the major LDL subclass) in monozygotic and dizygotic women twin pairs. The analysis is based on 203 monozygotic and 145 dizygotic pairs of adult female twins who participated in the second examination of the Kaiser Permanente Women Twins Study. The average age was 51 years at this exam and 90% were white. Concordance analysis revealed that monozygotic cotwins shared LDL subclass phenotypes more frequently than dizygotic cotwins, and this was confirmed using logistic regression analysis after controlling for potential confounding factors. Heritability analyses suggested that approximately one third to one half of the variation in LDL peak particle diameter, a continuous variable reflecting LDL size, could be attributed to genetic influences. Commingling analysis of the frequency distribution of LDL peak particle diameter identified three distinct subgroups of subjects, one of which corresponded to those subjects with LDL subclass phenotype B. This result could reflect the presence of a major gene effect on LDL size. These analyses in women twins demonstrate substantial genetic influences on LDL size heterogeneity, confirming previous studies in families. In addition, significant nongenetic factors are also apparently operating and thus could provide opportunities for targeted intervention to reduce coronary heart disease risk. (Arteriosclerosis and Thrombosis 1993;13:687–695)

KEY WORDS • cholesterol • coronary heart disease • genetics • heritability • LDL • twins

Low density lipoprotein (LDL) cholesterol is believed to contribute to the development of coronary heart disease.1-3 It has been recognized for a number of years, however, that LDL particles are heterogeneous in both size and density.4-6 Recently, two distinct LDL subclass phenotypes denoted A and B have been described in individual study subjects on the basis of gradient gel electrophoresis.7 LDL subclass phenotype A is characterized by a predominance of large, buoyant LDL particles, generally with a peak particle diameter greater than 255 Å and skewing of the gradient gel scan to the right. In contrast, subjects with LDL subclass phenotype B have a predominance of small, dense LDL particles with a diameter less than or equal to 255 Å and skewing of the gradient gel scan to the left. In studies to date, 85–90% of subjects have one of these two LDL subclass phenotypes, with the remainder having an intermediate phenotype. LDL subclass phenotype B is common, being present in approximately 25% of women in the general population.8 Several studies have shown that small, dense LDL is associated with increased risk of coronary heart disease.7,8,10 The most recent of these, which was based on angiographic evaluations, showed the prevalence of “small and very small” LDL particles to be 48% among coronary artery disease patients,10 similar to the 54% prevalence of LDL subclass phenotype B among male myocardial infarction survivors.7 Both of these studies and others have shown small LDL to be associated with a constellation of other lipoprotein-related risk factors,
including increased plasma triglyceride and apolipoprotein (apo) B and decreased high density lipoprotein (HDL) cholesterol and apo AI levels.\textsuperscript{11} The mechanism underlying the increased risk associated with LDL subclass phenotype B, however, is not yet understood.

Genetic influences on LDL subclass phenotypes have also been described. Two family studies, one in primarily healthy families and one in families with familial combined hyperlipidemia, have shown that the inheritance of LDL subclass phenotype B is consistent with the presence of a single, major gene effect with a dominant or additive mode of inheritance and a common allele frequency.\textsuperscript{9,12} A recent article from the National Heart, Lung, and Blood Institute (NHLBI) male twin study, using a different calibration method for LDL size, reported an unadjusted heritability estimate of 0.52.\textsuperscript{13} Using a candidate-gene approach, linkage analyses have shown that phenotype B is not linked to the apo A gene on chromosome 2.\textsuperscript{14,15} An initial report, however, has linked LDL subclass phenotypes to the LDL receptor gene on chromosome 19,\textsuperscript{16} lending further support to the presence of genetic influence.

Environmental and behavioral influences on LDL heterogeneity are also well documented. Age, menopausal status in women, and gender have been associated with variation in LDL subclasses.\textsuperscript{8,17} Similarly, penetrance estimates for phenotype B are reduced in young men and premenopausal women based on complex segregation analysis.\textsuperscript{8,12} Other important covariates appear to be use of \beta-adrenergic blockers,\textsuperscript{10} diabetes status and plasma insulin levels,\textsuperscript{18} central obesity,\textsuperscript{19} and dietary factors.\textsuperscript{20,21} Thus, the expression of LDL subclass phenotypes and their associated risk for coronary heart disease clearly involve both genetic and environmental components.

The purpose of the present analysis was to further evaluate the relative importance of genetic and nongenetic influences on LDL subclass phenotypes in a large, unique sample of nearly 700 women twins. Both the dichotomous LDL subclass phenotype classification and the LDL peak particle diameter, a continuous variable reflecting the size of the major LDL subclass, have been used. Several statistical approaches, each with different strengths and limitations, were employed to estimate the magnitude of genetic influence on LDL size heterogeneity. These approaches included concordance analysis, discordant pair analysis, heritability analysis, and commingling (or mixture) analysis.

### Methods

#### Study Subjects

The women twins in this study participated in the second examination of the Kaiser Permanente Women Twins Study in Oakland, Calif., during 1989–1990. The original sample consisted of 434 pairs of female twins born in 1960 or earlier who participated in the baseline visit during 1978–1979.\textsuperscript{22} At that time, zygosity was determined on the basis of 20 polymorphic loci, such that the probability of misclassification of a pair who were concordant on all these markers as monozygotic was less than 0.001. Eighty-one percent of the original sample returned for the second examination. Four pairs were excluded because of insufficient plasma for lipid analysis, for a total of 696 women in 348 twin pairs. Two hundred three pairs were monozygotic (MZ) and 145 were dizygotic (DZ). The median age of the women was 51 years and 90% were white. Because the distributions of LDL subclass phenotypes were similar, all ethnic groups were included in the present analysis.

Each participant had a physical examination and completed a medical history questionnaire, including items on menopausal status and medication use, including \beta-blocker use. Plasma glucose levels were measured in the fasting state and again 2 hours after a 75-g oral glucose load (Glutol, Paddock Laboratories, Minneapolis, Minn.) using the glucose oxidase method.\textsuperscript{23} Subjects who reported a physician’s diagnosis of diabetes and current use of insulin or oral hypoglycemic medication were classified as diabetic. The remaining subjects were classified as having diabetes, impaired glucose tolerance, or normal glucose tolerance on the basis of World Health Organization criteria.\textsuperscript{24} Eighteen subjects failed to either take or retain the glucose solution. Five of these subjects had fasting glucose values between 100 and 140 mg/dL and were classified as possible diabetics. The other 13 subjects with fasting levels below 100 mg/dL were considered to have normal glucose values. Four subjects in three pairs had no glucose values and were excluded from analyses that involved diabetes status. Similarly, two subjects with uncertain menopausal status and four subjects with unknown \beta-blocker use were excluded from analyses that involved these variables.

#### Laboratory Measurements

For the LDL subclass phenotype and LDL peak particle diameter determinations, 30 mL of fasting whole blood was drawn from each study subject, and plasma was immediately obtained by centrifugation. Gradient gel electrophoresis was performed on plasma using 2–16% polyacrylamide gradient gels (Pharmacia) as previously described.\textsuperscript{6,25} Two gradient gels were run for each subject, one using whole plasma and lipid stain (oil red O) and a second using isolated LDL and protein stain (Coomassie blue). The sizes of the major and minor LDL peaks were calculated on the basis of a calibration curve constructed from high-molecular-weight standards.\textsuperscript{6} The resulting scans were used to classify study subjects as having either LDL subclass phenotype A, phenotype B, or an intermediate phenotype (I)\textsuperscript{11} by three independent reviewers who were blinded to cotwin pairing, zygosity, and lipid levels. Disagreements were resolved by negotiation among the three reviewers.

In the present study, 89% of subjects could be classified into either phenotype A or B. The remaining subjects had an intermediate I phenotype, consisting of a gradient gel scan in which either the peak particle diameter was close to the 255-A cutpoint with little or no skewing of the curve or two distinct major peaks were observed. Because phenotype I was relatively common in this study (11%), it was considered as a separate category for the present analysis whenever possible. However, when dichotomous variables were needed, both the “broad” and “narrow” definitions of phenotype B were used.\textsuperscript{11} In the broad definition, phenotype I subjects were grouped with clear phenotype B subjects. In the narrow definition, phenotype I subjects were grouped with phenotype A subjects. Pre-
vious segregation analyses in families showed similar results for both definitions.

In addition to the dichotomous classification of LDL subclass phenotypes, the diameter of the major LDL subclass peak was used as a continuous variable in some analyses. Both variables reflect the size of LDL particles in an individual subject. The peak particle diameters reported here are based on the LDL gels with protein stain; all results were similar for diameters determined from whole-plasma gels using lipid stain.

**Genetic Analysis**

**LDL subclass phenotypes.** The similarity of LDL subclass phenotype in sisters was investigated by examining the number of twin pairs in which sisters had the same phenotype (concordant pairs) and the number of pairs in which sisters had different phenotypes (discordant pairs) by zygosity status, using each of the three possible phenotypes A, B, and I. Proband concordance ratios were determined by zygosity for both the broad and narrow definitions of LDL subclass phenotype B. The proband concordance ratio is defined as $2C/(2C+D)$, where C is the number of concordant phenotype B pairs and D is the number of discordant pairs. χ² tests comparing MZ and DZ phenotype frequencies used only the concordant phenotype B pairs and the discordant pairs. Because concordance ratios consider only pairs with at least one phenotype B cotwin per pair, the sample size is effectively decreased and the statistical power for comparing concordance of MZ and DZ twins for phenotype B is reduced. Therefore, multivariate logistic regression analysis was also used to assess the association between the LDL subclass phenotype of one cotwin and the phenotype of the other cotwin from the same pair by using all pairs in the sample. This approach allows the estimation of an odds ratio describing the magnitude of this association between cotwins and also allows covariates to be evaluated simultaneously. The cotwin whose phenotype was used as the dependent variable was selected randomly from each pair, and her covariates were used in the analysis. The analysis was performed separately for each zygosity to compare the magnitude of the odds ratios in MZ and DZ pairs. A statistical comparison of these odds ratios was performed using another model with a zygosity-by-phenotype interaction term. Because of the limited statistical power of interaction terms, a probability level of 0.10 was used for testing the significance of the interaction terms. Because logistic regression analysis requires a dichotomous outcome, this analysis was repeated using both the broad and narrow definitions of phenotype B.

Individual cotwins in MZ pairs discordant for LDL subclass phenotypes A and B were compared to further identify factors that might explain the occurrence of different phenotypes. For simplicity, twin pairs in which either cotwin had phenotype I were excluded from this analysis.

**Heritability analysis of LDL peak particle diameter.** To determine the proportion of variance in LDL peak particle diameter attributable to genetic influences, heritability analysis was performed using the analysis of variance model with the modifications proposed by Christian et al. Under the assumptions of this model, heritability is an estimate of the proportion of the variance in LDL peak particle diameter values attributable to genetic influences.

**Results**

**LDL Subclass Phenotypes**

The frequency of LDL subclass phenotypes in individual study subjects is shown in Table 1 by zygosity. Approximately 10% of all individual cotwins had LDL subclass phenotype B, while 79% had an LDL subclass

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>MZ (%)</th>
<th>DZ (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>320 (78.8)</td>
<td>227 (78.3)</td>
<td>547 (78.6)</td>
</tr>
<tr>
<td>I</td>
<td>46 (11.3)</td>
<td>31 (10.7)</td>
<td>77 (11.1)</td>
</tr>
<tr>
<td>B</td>
<td>40 (9.9)</td>
<td>32 (11.0)</td>
<td>72 (10.3)</td>
</tr>
</tbody>
</table>

Total 406 (100) 290 (100) 696 (100)

LDL, low density lipoprotein; MZ, monozygotic; DZ, dizygotic; I, intermediate.

Estimates near 0 imply no genetic effects, while values close to 1 imply strong genetic influence. For the present analysis, the “within-pair” estimate of heritability and the classical heritability estimate, defined as twice the difference of the intraclass correlations of MZ and DZ pairs, are reported. For all heritability analyses, the F tests of equality of total variances for MZ and DZ twins were not significant using a conservative α value of 0.28 except for the subgroup of postmenopausal pairs only (p=0.17).
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Regression requires a binary outcome, the analysis was
both cotwins were postmenopausal. There were 165
frequencies of concordant phenotype B pairs and discor-
dant variables. Because logistic re-
gression was used to assess the association between the
phenotype of one cotwin and the phenotype of the
other cotwin in the same pair, after incorporating
menopausal status, diabetes status, and β-blocker use as
potential confounding variables. Because logistic re-
gression requires a binary outcome, the analysis was
performed twice, first using the broad definition of LDL
subclass phenotype B and then the narrow definition
(see “Methods”). Odds ratios and 95% confidence
intervals for these analyses by zygosity are given in
Table 3 for the broad and narrow definitions of pheno-
type B.

In models 1 and 2, only LDL subclass phenotypes of
the cotwins were used (Table 3). The odds ratios reflect-
ing the association between one cotwin’s pheno-
type and the other cotwin’s phenotype were 18.5 and 6.5
for MZ and DZ pairs, respectively. Note that the 95%
confidence interval of the odds ratio for each zygosity
does not include the estimate for the other zygosity. In
addition, another model including an interaction term
for zygosity × phenotype demonstrated a statistically
significant difference (p = 0.09). Thus, the association
of LDL subclass phenotypes is greater in MZ pairs than in
DZ pairs. Similar results are seen in models 3 and 4, in
which menopausal status, diabetes status, and current
β-blocker use were included as covariates, although the
interaction term was not significant. Diabetes status
was also significant for both zygosities, but odds ratios
for LDL subclass phenotype did not change in either
zygosity.

Using the narrow definition of phenotype B, large
differences in the odds ratio for LDL subclass pheno-
type were again seen by zygosity: 10.5 for MZs (model
5) and 5.9 for DZs (model 6). However, similar to the
concordance results, these differences were not as sub-
stantial as those seen for the broad definition. In the
model including covariates, the odds ratio was some-
what reduced for MZ twins to 7.9 (model 7) but was still
highly significant, while menopausal and diabetes status
were also significant. For DZ twins, the odds ratio
actually increased to 8.8 (model 8), and β-blocker use
was a significant covariate.

**Discordant MZ pairs.** Because MZ cotwins are by
definition genetically identical, pairs discordant for
LDL subclass phenotype can provide clues to nonge-
etic influences. For this purpose, the 13 MZ discordant
pairs, i.e., those in which one cotwin had phenotype A
and the other had phenotype B, were examined for
pairs, i.e., those in which one cotwin had phenotype A
and the other had phenotype B, were examined for
menopausal status, β-blocker use, and diabetes status.
Eleven of the 13 pairs were discordant for menopausal
status (four premenopausal and seven postmeno-
apausal). Current use of β-blocker medication was also
not associated with LDL subclass phenotype discord-
cance in this sample (data not shown). Thus, neither
menopausal status nor β-blocker medication use ap-
ppears to explain the discordant MZ pairs.

In this sample of women twins, there was a strong
association between phenotype B and diabetes status.
That is, phenotype B was present in 6% of normal
subjects, in 22% of subjects with impaired glucose
tolerance, and in 37% of diabetic subjects. Among the
13 MZ pairs discordant for LDL subclass phenotype,
there were six pairs in which both cotwins were normal
and one pair in which both cotwins had impaired

glucose tolerance. However, there were five pairs in
which only the phenotype A twin was normal: in three
of these pairs the phenotype B cotwin had impaired

glucose tolerance and in two pairs the phenotype B
cotwin was diabetic. Thus, nongenetic aspects of dia-

TABLE 2. Concordance of LDL Subclass Phenotypes by Zygosity

<table>
<thead>
<tr>
<th>Concordant pairs</th>
<th>MZ pairs (%)</th>
<th>DZ pairs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/A</td>
<td>145 (71.4)</td>
<td>97 (66.9)</td>
</tr>
<tr>
<td>I/I</td>
<td>9 (4.4)</td>
<td>3 (2.1)</td>
</tr>
<tr>
<td>B/B</td>
<td>8 (3.9)</td>
<td>5 (3.5)</td>
</tr>
<tr>
<td>All concordant</td>
<td>162 (79.7)</td>
<td>105 (72.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Discordant pairs</th>
<th>MZ pairs (%)</th>
<th>DZ pairs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/I</td>
<td>17 (8.4)</td>
<td>18 (12.4)</td>
</tr>
<tr>
<td>I/B</td>
<td>11 (5.4)</td>
<td>7 (4.8)</td>
</tr>
<tr>
<td>A/B</td>
<td>13 (6.4)</td>
<td>15 (10.3)</td>
</tr>
<tr>
<td>All discordant</td>
<td>41 (20.2)</td>
<td>40 (27.5)</td>
</tr>
<tr>
<td>Total pairs</td>
<td>203 (100%)</td>
<td>145 (100%)</td>
</tr>
</tbody>
</table>

LDL, low density lipoprotein; MZ, monozygotic; DZ, dizygotic;
I, intermediate. Percentages may not add to 100% because of
rounding.

phenotype A and 11% had an intermediate phenotype.
These percentages were similar in MZ and DZ twins.
When only one randomly selected cotwin per pair was
considered, the results were again similar (data not
shown).

**Concordance of LDL subclass phenotypes.** For all
possible combinations of LDL subclass phenotypes in
twin sisters, the frequencies of concordant and discor-
dant pairs are shown in Table 2 by zygosity. Each type
of concordant pair (A in both cotwins, I in both cotwins,
and B in both cotwins) was more frequent among the
MZ pairs than among the DZ pairs, with A/A pairs
predominating. Overall, 80% of MZ pairs were concor-
dant, while 73% of DZ pairs were concordant. Using
the broad definition of LDL subclass phenotype B, the
proband concordance ratio was 0.65 for MZ and 0.48 for
DZ pairs. For the narrow definition, the proband con-
cordance ratio was 0.40 for MZs and 0.31 for DZs.
Comparison of frequencies of concordant phenotype B
pairs and discordant pairs for MZs and DZs was not
significant at the a = 0.05 level using the broad definition
(p = 0.08) and was not significant using the narrow
definition (p = 0.55).

Because LDL subclass phenotype B was considerably
more common in postmenopausal women than in pre-
menopausal women (16% versus 3%, respectively), the
concordance analysis was repeated for pairs in which
both cotwins were postmenopausal. There were 165
such pairs (47.4% of the sample), and concordance
ratios were higher than for the total sample: 0.73 and
0.58 for MZ and DZ pairs, respectively, using the broad
definition, and 0.50 and 0.38, respectively, using the
narrow definition. However, comparisons of the fre-
quencies of concordant phenotype B pairs and discor-
dant pairs in MZs and DZs were not significant using
either definition (p > 0.10).

**Logistic regression analysis.** Multivariate logistic
regression was used to assess the association between the
phenotype of one cotwin and the phenotype of the
other cotwin in the same pair, after incorporating
menopausal status, diabetes status, and β-blocker use as
potential confounding variables. Because logistic re-
gression requires a binary outcome, the analysis was
performed twice, first using the broad definition of LDL

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TABLE 3. Multivariate Logistic Regression Models Predicting LDL Subclass Phenotype in One Cotwin per Pair

<table>
<thead>
<tr>
<th>Broad definition of LDL subclass phenotype B</th>
<th>Model 1 MZ (n=196)</th>
<th>Model 2 DZ (n=141)</th>
<th>Model 3 MZ (n=196)</th>
<th>Model 4 DZ (n=141)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL subclass phenotype in other cotwin (0=A, 1=B)</td>
<td>18.5</td>
<td>6.5</td>
<td>18.5</td>
<td>5.9</td>
</tr>
<tr>
<td>(8.0, 43.2)</td>
<td>(2.6, 16.1)</td>
<td>(7.1, 47.2)</td>
<td>(2.2, 16.1)</td>
<td></td>
</tr>
<tr>
<td>Menopausal status (0=pre, 1=post)</td>
<td>...</td>
<td>...</td>
<td>2.5</td>
<td>2.0</td>
</tr>
<tr>
<td>(0.9, 7.0)</td>
<td>(0.6, 6.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes status (0=normal, 1=IGT or diabetic)</td>
<td>...</td>
<td>...</td>
<td>4.4</td>
<td>5.1</td>
</tr>
<tr>
<td>(1.6, 12.3)</td>
<td>(1.7, 15.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current β-blocker use (0=no, 1=yes)</td>
<td>...</td>
<td>...</td>
<td>0.6</td>
<td>2.9</td>
</tr>
<tr>
<td>(0.1, 3.1)</td>
<td>(0.7, 12.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zygosity x phenotype*</td>
<td>2.8† (0.8, 9.8)</td>
<td>2.8 (0.8, 10.7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Narrow definition of LDL subclass phenotype B</th>
<th>Model 5 MZ (n=196)</th>
<th>Model 6 DZ (n=141)</th>
<th>Model 7 MZ (n=196)</th>
<th>Model 8 DZ (n=141)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL subclass phenotype in other cotwin (0=A, 1=B)</td>
<td>10.5</td>
<td>5.9</td>
<td>7.9</td>
<td>8.8</td>
</tr>
<tr>
<td>(3.5, 31.4)</td>
<td>(1.7, 20.6)</td>
<td>(2.2, 28.8)</td>
<td>(1.9, 40.8)</td>
<td></td>
</tr>
<tr>
<td>Menopausal status (0=pre, 1=post)</td>
<td>...</td>
<td>...</td>
<td>10.1†</td>
<td>1.6</td>
</tr>
<tr>
<td>(1.2, 84.7)</td>
<td>(0.3, 9.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes status (0=normal, 1=IGT or diabetic)</td>
<td>...</td>
<td>...</td>
<td>4.2‡</td>
<td>5.1‡</td>
</tr>
<tr>
<td>(1.3, 13.6)</td>
<td>(1.3, 20.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current β-blocker use (0=no, 1=yes)</td>
<td>...</td>
<td>...</td>
<td>1.1</td>
<td>11.7§</td>
</tr>
<tr>
<td>(0.2, 5.3)</td>
<td>(2.2, 62.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zygosity x phenotype*</td>
<td>1.8 (0.3, 9.5)</td>
<td>1.2 (0.2, 7.8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LDL, low density lipoprotein; MZ, monozygotic; DZ, dizygotic; IGT, impaired glucose tolerance.
*Based on a model including both MZ and DZ pairs.
†p<0.10, ‡p<0.05, §p<0.01, ¶p<0.001.

**LDL Peak Particle Diameter**

Figure 1 shows the distribution of LDL peak particle diameter by zygosity, and descriptive statistics are given in Table 4. The distributions for individual MZ and DZ twins were very similar, as reflected by the mean values of 267.6 Å and 267.0 Å, respectively. Note that the overall distribution is skewed to the left (skewness=−0.82). Skewness values within phenotypes were, however, closer to 0 than the overall skewness value (Table 4).

**Heritability analysis.** For comparison with a recent report based on the NHLBI male twin study,13 heritability analyses were performed in this sample of women twins.26 For all twin pairs, the intraclass correlations for MZ and DZ pairs were 0.71 and 0.44, respectively. The resulting classical heritability estimate was 0.54 (p<0.001), and the within-pair estimate was similar (0.48, p<0.001; Table 5). Thus, approximately 50% of the variability in LDL peak particle diameter appears to be due to genetic influences.

This analysis was repeated using only postmenopausal pairs. Although the sample size was reduced from a total of 348 pairs to 165 pairs, the results were similar: the intraclass correlation for MZs was 0.73 and for DZs was 0.46 (Table 5). The classical heritability estimate was also similar, at 0.55 (p=0.003), although the within-pair estimate was slightly lower (0.34, p=0.02). Heritability was estimated again using only pairs in which neither twin was diabetic, in pairs in which neither twin was currently using β-blocker medication, and in Caucasian pairs. Heritability estimates were similar in all these subgroups, ranging from 0.35 to 0.52.

**Commimgling analysis.** In contrast to many biological traits, the distribution of LDL peak particle diameter is skewed to the left (Figure 1). The overall skewness
value was −0.82 and was similar for both MZ and DZ twins (Table 4).

Using all 696 twins, the bimodal model provided a significantly better fit to the frequency distribution than a unimodal model (Table 6, model 2 versus model 1, \( p<0.001 \)). The mean values of the two subdistributions in the bimodal model were 251 Å and 269 Å. However, a trimodal model provided a significantly better fit to the data than the bimodal model (model 3 versus model 2, \( p<0.01 \)), with mean values of 251 Å, 267 Å and 274 Å (Figure 2A). The relative areas for each subdistribution were 0.11, 0.64, and 0.25, respectively (Table 6). The sum of the fitted subdistributions is shown in comparison with the observed distribution in Figure 2B, and the fitted distributions provide a reasonable fit to the observed data. In particular, the skewing to the left of the observed distribution is well represented by the left tail of the fitted distributions. Fitting four subdistributions did not significantly improve the fit of the model (\( p>0.20 \)).

Because cotwins in a pair are not independent observations, the commingling analysis was repeated using one randomly selected cotwin per pair. The results were very similar, with mean values of 250 Å, 268 Å, and 274 Å for the three subdistributions (data not shown). The analysis was also performed for postmenopausal women only (\( n=387 \)), and mean values of the subdistributions were again similar (252 Å, 264 Å, and 272 Å).

Finally, the three subdistributions were compared with the LDL subclass phenotype classifications in Figure 2C. The subjects with phenotype B corresponded almost completely to the lowest subdistribution with a mean value of 251 Å, indicating that this phenotype apparently corresponds to a distinct subgroup of individuals. Those with LDL subclass phenotype A were distributed in the two upper subdistributions. The subjects with phenotype I were primarily in the lower third of the middle distribution and thus do not correspond to the subdistributions as identified in the commingling analysis.

### Discussion

The results of these analyses based on women twins confirm previous reports of both genetic and nongenetic influences on LDL subclasses. MZ twins were more concordant than DZ twins when LDL subclass phenotype classifications were used, although the difference was not statistically significant. This relatively small difference is consistent with the proposed dominant mode of inheritance for LDL subclass phenotype B, and known environmental and behavioral influences on this trait. That is, concordance in MZ twins is expected to be only twice that in DZ twins under a dominant mode of inheritance, even when the trait is completely penetrant. In contrast, for example, under a recessive mode of inheritance a fourfold difference is expected.26

### Table 5. Heritability Analysis of LDL Peak Particle Diameter

<table>
<thead>
<tr>
<th></th>
<th>No. of pairs</th>
<th>Intraclass correlations</th>
<th>Heritability estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MZ</td>
<td>DZ</td>
<td>( \sigma_{MZ} )</td>
</tr>
<tr>
<td>All pairs</td>
<td>203</td>
<td>145</td>
<td>0.709</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>93</td>
<td>72</td>
<td>0.733</td>
</tr>
<tr>
<td>Nondiabetic</td>
<td>180</td>
<td>123</td>
<td>0.641</td>
</tr>
<tr>
<td>Pairs not using</td>
<td>186</td>
<td>125</td>
<td>0.675</td>
</tr>
<tr>
<td>( \beta )-blocker medication</td>
<td>183</td>
<td>131</td>
<td>0.702</td>
</tr>
</tbody>
</table>

LDL, low density lipoprotein; MZ, monozygotic; DZ, dizygotic.
TABLE 6. Commingling Analysis of LDL Peak Particle Diameter

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>μ1</td>
<td>267.3</td>
<td>...</td>
<td>251.2</td>
</tr>
<tr>
<td>μ2</td>
<td>269.3</td>
<td>267.2</td>
<td></td>
</tr>
<tr>
<td>σ1</td>
<td>7.8</td>
<td>4.5</td>
<td>4.4</td>
</tr>
<tr>
<td>σ2</td>
<td>5.7</td>
<td></td>
<td>4.9</td>
</tr>
<tr>
<td>σ3</td>
<td></td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>f1</td>
<td>1.00</td>
<td>0.11</td>
<td>0.11</td>
</tr>
<tr>
<td>f2</td>
<td>...</td>
<td>0.89</td>
<td>0.64</td>
</tr>
<tr>
<td>f3</td>
<td>...</td>
<td></td>
<td>0.25</td>
</tr>
<tr>
<td>ln L</td>
<td>-1,782.94</td>
<td>-1,732.21</td>
<td>-1,726.30</td>
</tr>
</tbody>
</table>

Comparison model

χ² (df) | 101.46 (3) | 11.82 (3) |

p | <0.001 | <0.010 |

LDL, low density lipoprotein; μ, mean; σ, standard deviation; f, frequency; L, likelihood.

Environmental factors that influence LDL subclass phenotypes10,17-21 would be expected to further reduce concordance. Matched cotwin logistic regression analysis demonstrates a very strong association between LDL subclass phenotypes in MZ cotwins (odds ratio of 18, model 3 in Table 3) and a significant but lower associ-

ation in DZ twins (odds ratio of 6, model 4) even after adjusting for covariates. Therefore, both analyses based on LDL subclass phenotype classifications are consistent with genetic influences.

Heritability analyses using LDL peak particle diameter as a measure of the size of the major LDL subclass also support the presence of genetic influences. Heritability estimates based on all twins were approximately 0.5 (p<0.001). These estimates do not change substantially when the analysis is restricted to postmenopausal pairs, to nondiabetics, or to subjects not using β-blocker medication, ranging from 0.34 to 0.5 and remaining statistically significant (p<0.03 for all estimates). Thus, between one third and one half of the variability in LDL size appears to be attributable to genetic influences in this sample of women twins.

However, these heritability values should be interpreted with caution, since the estimates assume a single, underlying gaussian distribution. Although this assumption was not met in the present data set (Table 6 and Figure 1), the heritability analysis in the women twins was performed for comparison with data from the third examination of the NHLBI study of older male twins. That study found similar heritability values for "LDL type" (0.52 unadjusted, p=0.12; 0.39 adjusted, p=0.41) based on a weighted estimate of the LDL size.13 Unlike the present study, those estimates were not statistically...
significant, largely due to the use of the “among-component” estimate of heritability, which is substantially less statistically powerful than other estimators.37

Because heritability analysis also assumes an underlying polygenic model, it cannot be used to detect major gene effects. In contrast, commingling analysis can provide preliminary evidence for the influence of a single, major genetic locus.39 Based on the distribution of LDL peak particle diameter, the presence of a distinct subgroup of subjects with a mean value of 251 Å was found, corresponding almost perfectly to those subjects with LDL subclass phenotype B (Figure 2C). This result is consistent with a single-gene inheritance based on complex segregation analysis previously seen in two samples of families.8,12

The presence of trimodality in the peak particle diameter distribution was an unexpected result. Phenotype A subjects are evenly distributed throughout the upper two subdistributions, with mean values of 267 and 274 Å. It is tempting to speculate that the three subdistributions represent genotype BB homozygotes, AB heterozygotes, and AA homozygotes, respectively. Interestingly, the phenotype I subjects fall primarily in the lower portion of the middle subdistribution. The commingling analysis in this sample, then, did not identify a subdistribution that corresponds to these intermediate phenotypes. The phenotype I subjects might represent partial penetrance of the proposed B allele, variable expressivity, or even the presence of a third allele at the proposed locus.

It is also possible that more complex genetic mechanisms, not considered in the present analyses, underlie LDL subclasses. For example, in addition to likely genetic–environmental interactions, there may be more than one major gene locus involved (locus heterogeneity) or even gene–gene interactions (epistasis). These more complex genetic models will need to be considered in future studies of this phenotype.

Although these analyses demonstrate that LDL subclass phenotypes are largely genetically influenced, other factors must also contribute to the variability in LDL particle size. As a result, there are important public health implications. Intervention to reduce coronary heart disease risk may be possible, especially if targeted to those subjects with small, dense LDL. Control of diabetes and glucose intolerance or insulin resistance16,38 may be important interventions, since diabetes status was the strongest nongenetic correlate of phenotype B in this sample.

The underlying mechanisms leading to the association of LDL subclass phenotype B with risk of coronary heart disease are not yet established. It is possible, for example, that small LDLs are more easily deposited in atheromas than larger LDLs. Although small LDL must carry relatively less cholesterol than large LDL, the deposition of more LDL particles could contribute to accelerated atherosclerosis. On the other hand, LDL subclass phenotype B may be primarily a marker for a constellation of well-recognized lipoprotein-related risk factors. A number of studies have demonstrated that small LDL is simultaneously associated with increased levels of plasma triglyceride and apo B and decreased HDL cholesterol (specifically HDL2) and apo A1.7,10,41 A detailed analysis of these associations is presented elsewhere.41 Thus, LDL subclass phenotype B may be a qualitative trait representing a common, atherogenic lipoprotein profile.

Most recently, it has been proposed that small LDL may be more susceptible to oxidation, at least in vitro.39–42 Because accumulating evidence indicates an important role for oxidative modification of lipoproteins in atherosclerosis,43,44 this is another potential mechanism underlying the role of small LDL in coronary heart disease risk. How genetic influences may be involved in these potential atherogenic mechanisms remains to be elucidated.

In conclusion, genetic analyses of LDL subclass phenotypes and LDL peak particle diameter in a large sample of women twins demonstrate substantial genetic influences on LDL size heterogeneity. In addition, significant nongenetic factors are also apparently operating, providing opportunities for targeted intervention to reduce the risk of coronary heart disease in genetically susceptible individuals.

Acknowledgments

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